Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE VIRULENCE OF DIPHTHERIA BACILLI FROM DIPHTHERIA PATIENTS AND DIPHTHERIA-CARRIERS *

GEORGE H. WEAVER

From the Memorial Institute for Infectious Diseases, Chicago

The study here reported began in the testing for virulence of cultures from diphtheria-bacillus-carriers as they were met in Durand Hospital. It was extended to include cultures from diphtheria patients taken early in the disease in order that comparison might be made with later cultures if the bacilli should persist unduly long, and also cultures from diphtheria patients who had harbored the bacilli so long that they might properly be classed as carriers. The carriers were admitted as such or were nurses on duty in the hospital. They did not give histories of diphtheria and had been detected as carriers in routine examinations. In some of these it was impossible to exclude very mild attacks of diphtheria at times more or less remote from the moment at which they came under observation. It is probable that nearly all of them were contacts, tho this could not be determined in regard to most of them from the facts available. The carriers among the nurses were detected in routine examination of the throat and nose. Negative Schick reactions showed all of them to be immune at the time, either naturally or as the result of the administration of diphtheria antitoxin. In fact, all the carriers gave negative Schick reactions with the possible exception of two encountered early in the study on whom this test was not made. My purpose was to determine what proportion of the bacilli which are recognized as diphtheria bacilli in the routine work are virulent. I made no effort to measure the degree of virulence of the individual cultures.

TECHNIC

Cultures from the throat and nose on Loeffler's serum medium were incubated about 20 hours. From these cultures, by repeated plating, pure cultures were secured and studied as to their morphology and cultural peculiarities and tested for their pathogenic properties in guinea-pigs. Usually but one pure culture was tested in each instance.

All the cultures were of the granular or barred types, corresponding with Types A, A′, C, C′, and D of Wesbrook, Wilson, and McDaniel.[1] In the

---

* Received for publication November 8, 1916.

[1] Rep. Minnesota State Bd. of Health and Vital Statistics, 1901, p. 613.

cultures usually a few and often very numerous short forms were associated with the predominating longer and medium-length ones. Most of the cultures after 20 hours on Loeffler's medium showed abundant granules with Neisser's stain, but this was inconstant, no granules being observed in 3 typical virulent strains.

In litmus milk all the cultures produced a faint reddening, and in dextrose litmus broth (Smith's sugar-free broth 1% dextrose) acid was produced by all save one. The last grew scarcely at all in this medium, but when ascites fluid was added, it grew well and produced acid. In deep stab cultures in dextrose agar all grew to the bottom of the puncture and none spread over the surface.

In testing the virulence of cultures, a 24-hour growth on a slant of Loeffler's medium was used. The cutaneous test was employed for 55 cultures, the subcutaneous test for 35 cultures, and both tests for 26 cultures. When both tests were used, the results corresponded.

In the cutaneous tests 2 guinea-pigs were used, one serving as a control. The control received 50 units of diphtheria antitoxin intraperitoneally 24 hours before inoculation with the cultures. Both guinea-pigs were inoculated intracutaneously with a sharp, flat-pointed needle, slightly bent near the end after the method of Riemsdijk.[2] Four or 6 cultures were usually tested on 1 guinea-pig. A culture was classed as virulent if a local reaction appeared in the test animal after 24 hours while the control showed none.

In subcutaneous tests the control received 25 units of diphtheria antitoxin intraperitoneally 24 hours before inoculation. Both the control and test animals received from one-eighth to one-fourth of the 24-hour growth on the surface of a slant of Loeffler's medium subcutaneously in the abdominal wall. The culture was classed as virulent if the control remained well while the test animal died showing the typical lesions, including local exudation, edema, and hyperemic adrenals.

Corresponding tests were carried out by both methods, with typical cultures of Hofmann's bacillus and cultures resembling diphtheria bacilli morphologically, but differing from them in growth in litmus milk, dextrose broth, and dextrose agar (diphtheroid bacilli). None of these cultures was fatal for guinea-pigs, but a few after intracutaneous inoculation caused slight local reactions in both test and control animal.

All cultures from patients with diphtheria and from carriers were virulent. Nonvirulent diphtheria bacilli were not found.

The cultures came from 36 patients with typical diphtheria at varying intervals after the onset of the disease, and from 14 carriers. In 31 of the cases of diphtheria a single culture was tested. These cases are recorded in Table 1. The number of days elapsing between the time when the culture was obtained and the time when the bacilli disappeared from the throat or nose, is indicated in each case. It will be noticed that the tested culture was sometimes secured shortly before cultures became negative. In 5 cases, 2 or more cultures were studied. These are recorded in Table 2. In each case the later cultures corre-

---

[2] Folia Microbiologica, 1915, 4.

TABLE 1

PERSISTENCE OF VIRULENT DIPHTHERIA BACILLI IN PATIENTS WITH DIPHTHERIA

Culture No.	Source of Culture	Day of Disease on Which the Tested Culture Was Made	Number of Days Before Subsequent Cultures Became Negative
21	Throat	1st	11
10	Throat	2nd	19
13	Throat	2nd	16
24	Throat	2nd	2
41	Nose	2nd	9
47	Throat	3rd	2
69	Throat	3rd	6
23	Nose	4th	17
28	Throat	4th	20
33	Throat	4th	17
40	Throat	4th	21
39	Throat	4th	5
59	Throat	4th	19
51	Throat	4th	1
52	Throat	5th	Died
2	Throat	6th	Died
25	Throat	6th	32
31	Throat	7th	23
48	Laryngeal tube	7th	No positive cultures from throat
29	Laryngeal tube	8th	No positive cultures from throat
63	Nose	8th	26
7	Throat	11th	9
60	Nose	11th	42
49	Nose	13th	4
3	Throat	14th	17
27	Throat	14th	10
70	Throat	27th	5
42	Throat	28th	8
5	Throat	29th	1
4	Throat	34th	14
19	Throat	36th	12

TABLE 2

PERSISTENCE OF VIRULENT DIPHTHERIA BACILLI IN PATIENTS WITH DIPHTHERIA

Culture No.		Source of Culture	Day of Disease on Which the Tested Culture Was Made	Number of Days Before Subsequent Cultures Became Negative
Case 1	50	Throat	1	4
	57	Throat	2	3
Case 2	37	Throat	2	25
	44	Throat	21	6
Case 3	32	Throat	2	32
	35	Nose	13	21
	45	Throat	32	4
Case 4	15	Right ear	4	39
	16	Nose	5	38
	20	Left ear	38	5
Case 5	53	Throat	7	19
	61	Throat	25	1

sponded in every way with the earlier ones and were uniformly virulent.

There was no evidence in the results secured in the study of the diphtheria patients to indicate that the bacilli become nonvirulent after remaining a considerable time on the infected surface, even when tested shortly before their final disappearance.

The 14 carriers of diphtheria bacilli are enumerated in Table 3.

TABLE 3

PERSISTENCE OF VIRULENT DIPHTHERIA BACILLI IN DIPHTHERIA-BACILLUS-CARRIERS

Case No.	Culture No.	Source of Culture	Days After Entrance Before Tested Culture Was Made	Number of Days Before Subsequent Cultures Became Negative
1	1	Throat	7	1
2	64	Throat	1	3
3	30	Throat	1	4
4	38	Throat	1	4
5	17	Nose	6	5
6	43	Throat	2	7
7	65	Throat	1	9
8	12	Throat	1	12
9	58	Throat	1	12
10	14	Throat	1	14
11	67	Nose	2	39
12	45	Nose	2	72
	46	Nose	34	40
	54	Nose	58	16
13	66	Throat	1	53
	68	Throat	47	7
14	71	Nose	7	
	73	Nose	84	
	74	Nose	129	

Three were nurses on duty in Durand Hospital. The remaining 11 were admitted as carriers, usually having been detected as such in routine bacteriologic examinations by the Chicago health department. None of these gave a history of having had diphtheria, altho some had had slight sore throat or "cold in the head" some days or weeks previously. Three had had a purulent nasal discharge. Most of these carriers got rid of the bacilli in a short time. In 3, positive cultures were very persistent, and there was no evidence during a long period of observation that the bacilli were becoming nonvirulent.

The conclusions reached in this study agree very well with those of previous workers, with whom the general observation has been that cultures from cases of clinical diphtheria are almost always virulent.

In the virulence tests the culture to be injected should show a vigorous growth, and therefore a favorable medium should be selected. This is emphasized by the experience of Williams,[3] who found that some of the segmented

[3] Jour. Med. Research, 1902, 8, p. 83.

varieties of the diphtheria bacillus grew scantily in broth, and that such cultures, except in large amounts, gave little reaction in guinea-pigs, but that when grown in ascites broth, in which they grew rapidly and abundantly, they showed a high degree of toxicity. Some of my cultures grew very slowly and poorly in broth, even after repeated transfers, but grew well on Loeffler's serum medium. Satisfactory results followed the injection of young cultures on Loeffler's medium, preceded in the control animal by a relatively large dose of diphtheria antitoxin.

The prevalence of carriers of diphtheria bacilli has been the subject of much study. Graham-Smith[4] has tabulated the results of a large number of observers on the infection of healthy contacts with diphtheria bacilli. These statistics show that of close contacts — members of infected families, relatives, and attendants — 36.6% are liable to become infected, that in hospital wards and institutions 14% are liable, and among scholars of infected schools, 8.7%. In Durand Hospital biweekly cultural examinations are made of the throats of nurses on duty. During the past 2 years, of 66 nurses, 10 became carriers, or 15.15%. None of these developed diphtheria. Goldberger, Williams, and Hachtel[5] undertook extensive examinations to determine the number of diphtheria-carriers among the general population of Detroit. Of 4093 apparently healthy persons without history of contact, 38 (0.928%) harbored morphologically diphtheria bacilli. Of 19 cultures isolated, 2 were virulent, suggesting a possible 0.097% of the individuals examined as carriers of virulent diphtheria bacilli. The remaining 17 cultures correspond with what are designated nonvirulent diphtheria bacilli.

This wide distribution of diphtheria bacilli in apparently healthy persons renders the relationship between virulent and nonvirulent strains of great practical importance, but the relationship is not entirely clear. Several times it has appeared to have been shown that nonvirulent strains of diphtheria bacilli had been secured from virulent ones, but it has not been possible to convert a nonvirulent into a virulent form. Most of the persons who have considered this matter from a practical standpoint have reached the conclusion that carriers of nonvirulent diphtheria bacilli are not a source of danger to others, and that such persons should not be subject to isolation. It has been a matter of much interest to know whether diphtheria bacilli in the throat and nose lose their virulence after prolonged residence. After reviewing the available data on this point, Graham-Smith[4] reaches the conclusion, held by most of those who have investigated the subject, that in a few instances the bacilli appear to have lost virulence to some extent, but that in the great majority they have retained their full virulence up to the time of their final disappearance. As stated, I have been unable to discover, either in carriers or in persons who have had diphtheria, a loss in virulence on the part of the bacilli after a prolonged residence in apparently healthy noses and throats, even tho they were secured a short time before their final disappearance.

---

[4] The Bacteriology of Diphtheria, 1908, p. 186.

[5] Bull. 101, Hyg. Lab., Washington, D. C., p. 29.

Recent observations from several sources have emphasized the fact that persistent carriage of diphtheria bacilli usually depends on some local condition, such as diseased tonsils, adenoids, or sinuses. In such cases the bacilli escape in small numbers, except periodically, when they may appear on the surface in abundance. While such cases are probably less dangerous as spreaders of infection than recent cases of diphtheria with abundance of infective secretions, still they must be considered as potential agents for dissemination of the disease and must be isolated until the bacilli have been shown to be nonvirulent.

CONCLUSIONS

Diphtheria bacilli from patients with diphtheria and from carriers who have been in contact with such patients are practically always virulent.

The bacilli in such cases usually remain virulent up to the time of disappearance, even tho a long time has elapsed.

Carriers should be kept in isolation until the bacilli have been gotten rid of, or until the strains cultivated have been proved to be nonvirulent.

Cultures from the nose, as well as from the throat, should be made in all suspected cases.